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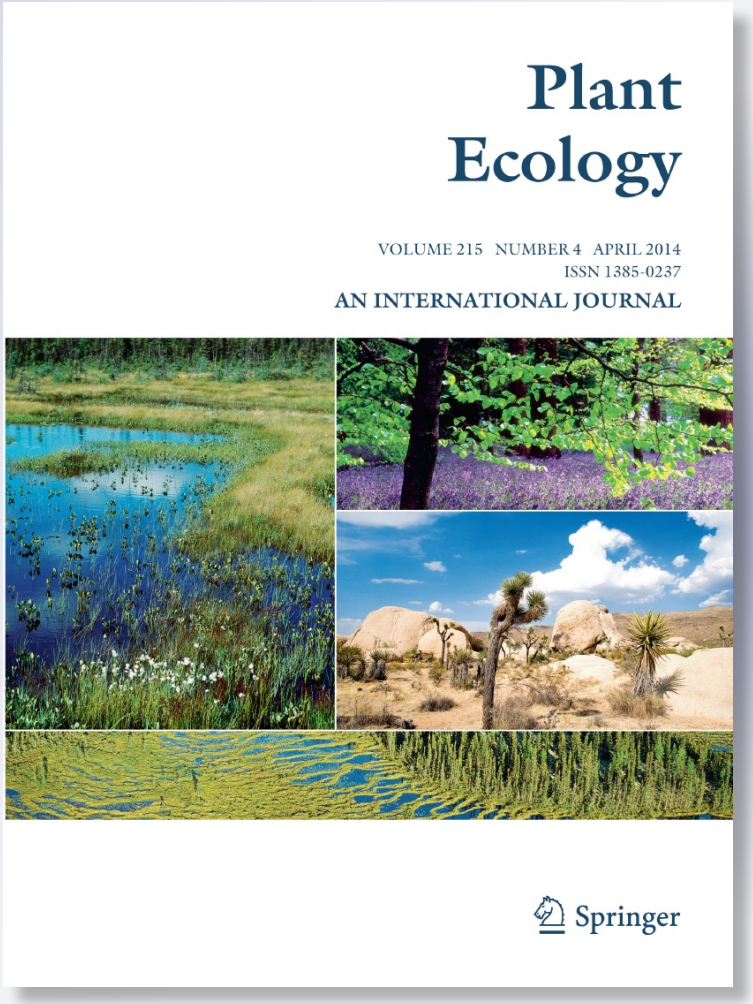

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Abstract Generalist pathogens can exhibit differential success on different hosts, resulting in complex host range patterns. Several factors operate to reduce realized host range relative to potential host range, particularly under field conditions. We explored factors influencing host range of the naturally occurring generalist ascomycete grass seed pathogen *Pyrenophora semeniperda*. We measured potential host range in laboratory experiments at high inoculum loads with 26 grass species, including the primary host *Bromus tectorum*, and developed models to predict susceptibility and tolerance based on host traits, including germination speed, seed hardness, seed size, and phylogenetic relations. We also examined pathogen and host density effects on infection and mortality. All species tested were at least somewhat susceptible to the pathogen at high inoculum loads, but both infection and mortality varied widely. Species more closely related to the original host (*B. tectorum*) were more susceptible to infection, whereas species with slower germination were less tolerant and therefore more likely to suffer mortality. Infection and mortality were sharply reduced as inoculum load was reduced. Intermediate loads had major negative impacts on dormant *B. tectorum* seeds but generally minimal effects on native species. In addition, field seed bank studies determined that *P. semeniperda* rarely exploits native grass species as hosts. This marked reduction in realized host range relative to potential host range indicates that laboratory host range studies are potentially a poor predictor of either the current or possible future realized host range for wildland plant pathogens.

Keywords *Drechslera campanulata* · Germination rate · Pathogen resistance · Pathogen tolerance · Phylogenetic distance · Plant–microbe interactions

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Introduction

The traditional paradigm of tightly coupled host–pathogen relations (Thompson and Burdon 1992) has

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recently been expanded into a broader framework, based on the recognition that there is a continuum of pathogen host specificity (Woolhouse et al. 2001; Barrett et al. 2009). For example, pathogens can range from highly specialized parasites to generalists that attack a wide range of host species or families. Specialization may be described qualitatively, as with a list of species that can be hosts, or quantitatively, where the pathogen has been demonstrated to have differential impacts on different hosts. Our collective scientific knowledge of generalist pathogens of wildland ecosystems is rather limited, but it is increasing as we study generalist pathogens in temperate forest systems (Maloney et al. 2005), tropical forests (Gilbert et al. 2007), and meadow grasslands (Schafer and Kotanen 2004). For a generalist pathogen, host range is a key element determining its ecological and evolutionary impact.

Pathogen host range can be characterized by both potential host range and actual, realized host range. The typical laboratory host range study measures the intrinsic, physiological ability of the pathogen to impact and utilize various hosts under optimum conditions for disease development at high inoculum loads, and therefore measures potential or maximum host range (e.g., Boedo et al. 2012). Laboratory host range testing is a necessary first step in the evaluation of host range. In contrast, the ability of the pathogen to utilize different hosts under field conditions is a better measure of its realized host range. Understanding how a plant pathogen is likely to operate under field conditions requires inclusion of extrinsic factors, such as variation in pathogen density, host density and environmental conditions (e.g., Gilbert et al. 2007).

We focused our investigations on factors affecting potential and realized host range of the seed pathogen *Pyrenophora semeniperda* (Brittlebank and Adam) Shoemaker. *P. semeniperda* is a naturally occurring ascomycete fungus that has been found primarily in the seed banks of *Bromus tectorum* L. (cheatgrass, downy brome), an important weed of western North American rangelands (Meyer et al. 2007, 2008). *P. semeniperda* is a generalist that infects seeds of multiple hosts, primarily grass species (Medd et al. 2003). This pathosystem includes *P. semeniperda* (anamorph *Drechslera campanulata*), its host *B. tectorum,* and several potentially co-occurring host species, both native and introduced. We were especially interested in pathogen effects on a small suite of native perennial grasses that are dominant species in intact shrubland vegetation (i.e., *Pseudoroegneria spicata* (bluebunch wheatgrass), *Elymus elymoides* (bottlebrush squirreltail), *Hesperostipa comata* (needle-and-thread), and *Achnatherum hymenoides* (Indian ricegrass); West and Young 2000). *B. tectorum* is host to a diverse array of fungal pathogens. Of these, the seedling–infecting smut fungi *Ustilago bullata* and *Tilletia bromi* are known to form highly host-specific races that can attack not just a single species but often only a subset of resistance phenotypes within a species (Pimentel et al. 2000; Meyer et al. 2005). In contrast, seed pathogens of the genus *Fusarium* found in the soils of *B. tectorum* monocultures are apparently generalists that can attack multiple grass species (Meyer et al. 2014). The host range of crown- and root-infecting pathogens on *B. tectorum* (Grey et al. 1995) has not yet been explored.

Pyrenophora semeniperda often prevents germination and causes mortality of infected seeds, but it can also infect and sporulate on germinating seeds that later develop into healthy seedlings (Campbell and Medd 2003). Under this latter scenario, seed resources are only partially utilized by the pathogen, and the host, though susceptible, can be said to be tolerant to pathogen infection (Roy and Kirchner 2000). When infection causes seed mortality, seed resources are fully available to the pathogen, and the host is said to be intolerant to pathogen infection. Rapid germination for susceptible seeds increases escape from mortality and thus tolerance to infection even at high inoculum loads, while slow-germinating, susceptible seeds are intolerant to infection and suffer high mortality (Beckstead et al. 2007). Rapid germination is thus a trait likely to confer tolerance to infection in susceptible species. Many factors influence seed germination rate, including temperature and seed physiological status (i.e., dormancy status), but germination rate under defined conditions can be considered a species trait. Resistance, or the degree of susceptibility to pathogen infection, is also a species trait, but it is distinct from tolerance to infection. Resistance influences the likelihood of infection whether or not the seed germinates, whereas tolerance influences the likelihood that an infected seed will be killed. Thus, germination rate is one of many seed traits that likely determine the host range of this seed pathogen and that mediate differential impacts on different hosts.

Our study had two main goals: (1) to define the potential host range of *P. semeniperda* and determine

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host traits that influence degree of utilization by the pathogen, i.e., host susceptibility and tolerance, and (2) to examine, for a subset of native grasses, the degree to which realized host range is reduced relative to potential host range under more realistic laboratory and field scenarios.

The potential host range study followed the traditional inoculation approach, incorporating 26 species. We measured host range in terms of both susceptibility to infection and tolerance to infection (i.e., seed mortality). To relate host traits to species responses observed in the potential host range study, we regressed host resistance and tolerance to infection on host seed germination rate, seed hardness, seed size, and relatedness to the host species of origin (*B. tectorum* for pathogen strains used in the trials).

The realized host range studies included a laboratory experiment to determine the effect of inoculum load on host susceptibility and tolerance for the primary host and four native grasses. We also quantified field seed density and disease levels in the seed banks of three native grasses and *B. tectorum* in order to examine the role of host seed density on realized host range. These studies go beyond a qualitative list of species as the description of host range and instead provide a quantitative view that includes the dynamic nature of the differential impacts that make up the potential and realized host range of a generalist pathogen.

Materials and methods

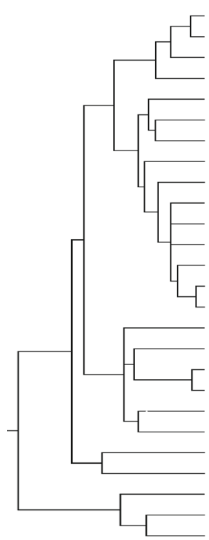
Potential host range

The purpose of this experiment was to determine the potential host range of *P. semeniperda* with regard to both resistance to infection and tolerance to infection, by testing the seeds of 25 co-occurring nontarget grass species as well as dormant and nondormant *B. tectorum* seeds (Table 1). Species selected included co-occurring annual grass weeds, introduced forage grasses, and native perennial grasses, including the four dominant species listed earlier. Seeds of each species had been stored under laboratory conditions for >6 months and were considered relatively nondormant. Dry conidial inoculum from two pathogen strains was used (collected from Tenmile Creek, Box Elder County, UT, USA and Whiterocks, Tooele County, UT, USA *B. tectorum* populations; see Meyer et al. (2010) for additional inoculum preparations). The two strains were not significantly different and were combined for analysis. These strains were selected at random from a newly obtained set of isolates by culturing surface-sterilized stromata from field-killed seeds found in *B. tectorum* seed banks. Fungi were cultured on V8 agar and then transferred to modified alphacell medium (MAM) agar, which promotes direct production of conidia on the mycelial surface. Conidia were harvested by rinsing the plates with sterile water, filtering the conidial suspension through a 25-μm sieve, and air-drying the filtrate. We scaled the dry weight of the conidial inoculum to the size of the seed to reduce inoculum waste, but insured that seed surfaces were saturated with conidia (e.g., seeds of similar size to *B. tectorum* received 0.003 g/50 seeds). Seeds and inoculum were placed in 4-ml glass vials and shaken vigorously. For each species, four replicates of 50 inoculated seeds (two for each strain) were placed in Petri dishes (100 × 15 mm) on moist germination blotters (Anchor Paper, St. Paul, MN, USA) and incubated at 20 °C under a 12-h diurnal photoperiod for 2 weeks followed by incubation without light (to reduce excessive seedling growth of germinated seeds) for 4 weeks. Dishes were watered as needed. Two replicates of 50 uninoculated seeds were included as controls.

Seed infection (presence of stromata) and mortality (presence of stromata on ungerminated seeds) were scored weekly. Germinated seeds were retained for the full 42 days with their coleoptiles clipped to quantify infection on germinated seeds. On day 42, remaining ungerminated seeds were scored as viable and dormant (as determined by cut test; Ooi et al. 2004), killed by the pathogen, or nonviable (initially nonviable or killed by a different pathogen).

As anticipated, the controls for this experiment contained low and infrequent levels of *P. semeniperda* background infection and seed mortality (average 6 % for infection and 2 % for mortality; mode zero for both infection and mortality). The majority of species experienced less than 10 % infection. The primary exceptions were *Koeleria macrantha* (37 %) and *P. spicata* (31 %). For these species, multiple strains of *P. semeniperda* were present within the inoculation treatments. Given the saturating inoculum level used in this experiment, we can assume that infection and mortality percentages would have been similar for

Table 1 Grass species phylogeny, nomenclature, and measured seed characteristics



Scientific name[a]	Code	Common name	Plant type	Mean germination time (days)[b]	Size (weight 25 seeds; g)[c]	Seed hardness[d]
Bromus tectorum	BRTE	Cheatgrass	Exotic weed	1.08	0.61	3
Bromus sterilis	BRST	Poverty brome	Exotic weed	1.18	0.02	2.5
Bromus inermis	BRIN	Smooth brome	Introduced forage	2.9	0.02	2
Bromus arvensis	BRAR	Japanese brome	Exotic weed	3.1	0.01	2.5
Secale cereale	SECE	Cereal rye	Exotic weed	1.03	0.05	4
Aegilops cylindrica	AECY	Jointed goatgrass	Exotic weed	3.05	0.98	2
Taeniatherum caput-medusae	TACA	Medusahead	Exotic weed	1.37	0.073	2.5
Agropyron cristatum	AGCR	Crested wheatgrass	Introduced forage	5.25	0.06	2
Elymus elymoides	ELEL	Squirreltail	Native grass	3.13	0.138	2
Pascopyrum smithii	PASM	Western wheatgrass	Native grass	3.9	0.07	3
Agropyron fragile	AGFR	Siberian wheatgrass	Introduced forage	2.8	0.1	2
Thinopyrum intermedium	THIN	Intermediate wheatgrass	Introduced forage	3.06	0.08	2
Pseudoroegneria spicata	PSSP	Bluebunch wheatgrass	Native grass	4	0.13	2
Elymus lanceolatus	ELLA	Thickspike wheatgrass	Native grass	3.05	0.08	2
Elymus alaskanus	ELAL	Slender wheatgrass	Native grass	--	0.08	2
Leymus cinereus	LECI	Basin wildrye	Native grass	5.4	0.13	2
Koeleria macrantha	KOMA	Prairie junegrass	Native grass	5.33	0.01	1
Festuca brevipila	FEBR	Hard fescue	Introduced forage	3.42	0.016	1
Festuca idahoensis	FEID	Idaho fescue	Native grass	3.75	0.142	2
Poa secunda	POSE	Sandberg bluegrass	Native grass	3.49	0.076	1.5
Ventenata dubia	VEDU	North Africa grass	Exotic weed	6.2	0.03	2
Hesperostipa comata	HECO	Needle and thread	Native grass	10.23	0.111	3
Achnatherum hymenoides	ACHY	Indian ricegrass	Native grass	--	0.086	4
Sporobolus cryptandrus	SPCR	Sand dropseed	Native grass	--	0.006	2
Bouteloua curtipendula	BOCU	Sideoats grama	Native grass	1.1	0.15	1
Pleuraphis jamesii	PLJA	James galleta	Native grass	2.59	0.341	2

Phylogeny illustrated to the left of the table is a pruned version of a chronogram originally based on 64 species. See Supplemental Methods A1 in the online supplemental materials for details

[a] Nomenclature based on USDA, NRCS. 2010. The PLANTS Database (http://plants.usda.gov, 28 September 2010). National Plant Data Center, Baton Rouge, LA, USA

[b] Mean germination time is the number of days to reach 50 % germination. If 50 % germination was not reached, then the number of days was left blank, and a value of 50 was used in the analysis

[c] Size was measured as the average weight of 25 seeds per species (g)

[d] Seed hardness was measured on a scale (1–4; soft-hard)

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these two species even without the relatively high background levels of *P. semeniperda*. Later inoculation treatments combined with surface sterilization of seeds of these two species confirmed this assumption. We used absolute values rather than control-corrected values (inoculum treatment − control treatment) in the graphical data presentation for these two species.

The effect of species and inoculum treatment on the proportion of seeds infected or killed was analyzed using analysis of variance (ANOVA) for a completely randomized design (PROC GLM; SAS Version 8.1, SAS Institute Inc., Cary, NC, USA). The response variables (proportions) were arcsine square-root transformed to improve homogeneity of variance prior to analysis, and predicted model residuals were checked for normality.

Host traits to predict potential host range

To predict which co-occurring hosts are likely to be impacted by this pathogen, we examined traits of 26 potential host species, including nondormant *B. tectorum* (Table 1). We quantified mean germination time, seed morphological traits (i.e., seed size and seed hardness), and phylogenetic relationships of host species. We then used data from the host range experiment to relate these traits to the ability of *P. semeniperda* to infect and kill host seeds. We measured host seed germination rate for the uninoculated control treatment in the host range experiment by counting germinated seeds on day 2, 4, 7, 11, 14, 21, and 28. Mean germination time (days to 50 % germination of viable seeds) was calculated by interpolation on germination time course curves. Seed size was determined by weighing three samples of 25 seeds from each bulk seed collection. Seed hardness was evaluated by cutting five seeds of each species and ranking them from soft to hard on a qualitative scale (Pringle et al. 2007).

To obtain measures of species relatedness among the 26 grass species used in our experiments, we constructed a phylogeny for a larger set of species from molecular data using a Bayesian inference analysis; the methods are described in full online (Supplemental Methods A1). The resulting chronogram was then used to estimate the phylogenetic distance between *B. tectorum* and all other species using function phydist in PHYLOCOM (Webb et al. 2008). Table 1 shows a pruned version of the chronogram for the 26 grasses.

To analyze which host traits explained variation in seed infection and mortality, we used a stepwise multiple regression analysis (mixed selection), with seed infection and mortality proportions from the host range experiment as dependent variables and phylogenetic distance, germination time, seed hardness, and seed size as the independent variables (JMP, Version 8.0.1, SAS Institute Inc., Cary, NC, USA). All percentages (proportions) were arcsine square-root transformed and remaining variables were log-transformed to attain normality of regression residuals.

Pathogen density effects to predict realized host range

To explore how the realized host range of this pathogen changes with inoculum load, we varied inoculum load on seeds of *B. tectorum* and four native hosts in a laboratory experiment that followed protocols similar to the potential host range experiment. The four native hosts included *A. hymenoides*, *E. elymoides, H. comata*, and *P. spicata*. These species offered a range of responses to *P. semeniperda* in the host range experiment. For the highest inoculum concentration, we used pure conidia (Whiterocks strain) sufficient to saturate the seed surface (0.003 g/50 seeds). Quantitatively reduced inoculum loads were obtained by diluting pure conidia with reagent grade talc (hydrated magnesium silicate) to obtain concentrations of 1:100, 1:200, 1:400, 1:800, 1:3,200, 1:12,800, and 1:51,200. This procedure is analogous to the typical procedure for quantifying relative inoculum levels using concentrations in liquid inoculum. Trial experiments determined that the talc had no effect on either seeds or pathogen. The response variables seed infection and mortality were determined as described above. There were four replicates of 50 seeds for each inoculum level (uninoculated control, pure conidia, and seven inoculum dilutions) for each of the five species.

This experiment was analyzed using analysis of covariance with inoculum concentration as the independent continuous variable, species as the independent class variable, and seed infection proportion and mortality proportion as the dependent variables (SAS Version 8.1, SAS Institute Inc., Cary, NC, USA). Dependent variables were arcsine square-root transformed to increase homogeneity of variance. Inoculum concentration was log transformed.

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Host and pathogen density in field seed banks

The seed bank study allowed us to examine the realized host range as it is currently expressed for cheatgrass and dominant native species and to also measure seed and pathogen density in field seed banks. Seed bank samples were obtained from plant communities dominated by three native grass species or by *B. tectorum* at mesic and xeric sites in Washington (Spokane and Saddle Mountain, respectively) and at a xeric site in Utah (Whiterocks; see Table 4 in Appendix). Seed bank samples were collected for 2 years at the Washington sites (2007 and 2008) and for 1 year at the Utah site (2007). To compare soil resources and soil physical traits, four soil cores were randomly collected and combined from each of the five study sites. Analyses were performed at the Brigham Young University Soils Laboratory, Provo, UT following the Association of Official Analytical Chemists (AOAC) methods (see Table 5 in Appendix for soil data). Soil samples were obtained from *B. tectorum* monocultures and areas of native grasses. We attempted to choose native communities where the influence of adjacent *B. tectorum* seed banks would be minimal. The three native species included *E. elymoides*, *H. comata*, and *P. spicata.*

To quantify seed density and disease incidence in native grass and *B. tectorum* seed banks at each location, we collected 20 samples (6 cm in diameter and 4 cm deep) in early autumn, after dispersal was complete but before any germination took place. *B. tectorum* seed banks were sampled randomly within a defined area. For perennial species, samples were taken from within the crowns of 20 randomly selected individuals. Seeds were identified to species, and intact and field-killed seeds (protruding stromata) were counted (Meyer et al. 2007). Intact seeds were then incubated for 4 weeks at 20 °C, then subjected to viability evaluation, to determine the number of seeds that were either viable or killed by the pathogen, as described earlier. Mean density was then calculated for viable and killed seeds of each species for each site and year of collection. Because of orders of magnitude differences in both viable and killed seed density between *B. tectorum* and native grass seed banks and the large number of zeroes in the native seed bank data, these data were not subjected to further statistical analysis.

Results

Potential host range

At saturating inoculum loads, seeds of all 26 species, including both dormant and nondormant *B. tectorum* seeds, were susceptible to infection by *P. semeniperda*, as evidenced by sporulation of the pathogen on seeds whether or not they germinated (40–99 %; Fig. 1a). There were significant differences in infection between inoculation treatments and among species (see Table 6 in Appendix for ANOVA). The magnitude of the inoculation treatment effect varied widely among species, leading to a highly significant species × inoculation treatment interaction. This indicates that species differed in degree of resistance to pathogen infection. The seven annual grass weeds sustained moderate to very high infection levels (0.53–0.98). The introduced forage species also had high proportions of infected seeds (>0.85). Eight of 14 native species had high levels of infection (>0.80), three had relatively low levels (<0.50), and the remainder exhibited moderate levels.

Not all species experienced *P. semeniperda*-caused mortality (Fig. 1b). There was again a highly significant difference between inoculation treatments and among species (see Table 6 in Appendix for ANOVA). The magnitude of the inoculation treatment effect again varied widely among the species, leading to a highly significant interaction effect for species × inoculation treatment. Only two of eight annual grass weeds (dormant *B. tectorum* and *V. dubia*) experienced significant increases in killed seeds with inoculation, suggesting that the other annual species have high tolerance to infection, at least in the nondormant state. Although all five introduced forage grass hosts had high infection levels (Fig. 1a), only one (*A. cristatum*) had high mortality, suggesting that the other forage grasses also had high tolerance to infection (Fig. 1b). Three of 14 native grass species (*L. cinereus*, *P. spicata*, and *K. macrantha*) experienced high levels of pathogen-caused death (>0.80), seven had mid-range levels (<0.65 but >0.30), and five had low mortality (<0.25).

This experiment showed that *P. semeniperda* has the ability, at high inoculum loads and under optimum conditions, to infect and utilize a wide range of grass hosts, but that some species were much more utilized than others. This was true both for partial utilization (infection and subsequent sporulation on germinated

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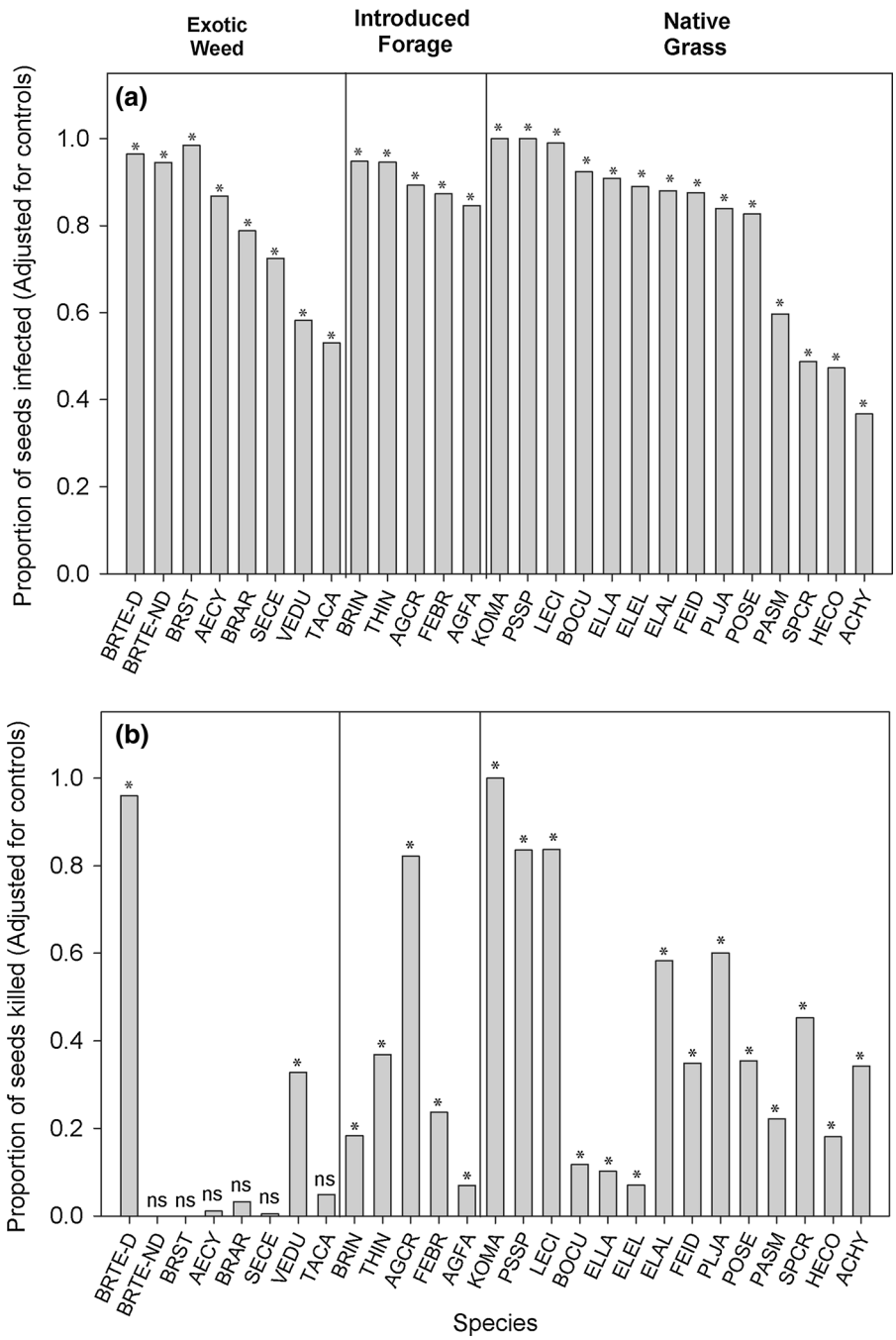

Fig. 1 Proportion of seeds **a** infected and **b** killed by *P. semeniperda* for 25 species that co-occur with the target species *B. tectorum* and for *B. tectorum* in two dormancy states (*D* dormant, *ND* nondormant; all means are adjusted for controls (e.g., inoculum treatment − control treatment). Species are grouped into functional categories: exotic annual weed, introduced perennial forage grass, and native perennial grass. Significance between inoculation treatment and control treatment for each species is indicated (ANOVA; $^{*}P < 0.05$ and $^{ns}P > 0.05$). See Table 1 for species codes and methods for information on controls

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Table 2 Results of stepwise multiple regression analyses of *P. semeniperda* seed infection and mortality on host traits (phylogenetic association, seed hardness, seed size, and germination time) for 25 co-occurring species and *B. tectorum* (nondormant only)

Dependent variables	Model			Independent variables							
				Phylogeny		Hardness		Size		Germination time	
	R^2	df	F	β	t	β	t	β	t	β	t
Seed infection	0.47	3, 25	6.42**	−0.02	−3.65***	−0.46	−3.33**	0.07	1.74^{ns}	Not loaded	
Seed mortality	0.42	2, 25	8.20**	Not loaded		−0.49	−2.72**	Not loaded		0.19	3.24**

"Not loaded" indicates a variable that was not selected in the multiple regression from the stepwise mixed selection procedure
Significance levels are designated in parentheses: ^{ns} $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

seeds) and for full utilization (seed mortality). Host range was considerably narrower when defined in terms of full utilization. Several species with high levels of infection (susceptibility) experienced little or no seed mortality because of their high levels of tolerance to infection. On the other hand, species that were relatively resistant often had mortality levels not much below infection levels, indicating high resistance but low tolerance to infection.

Host traits to predict potential host range

Stepwise multiple regression revealed that host traits explained significant variation in *P. semeniperda*-caused seed infection ($r^2 = 0.52$, $F = 5.61$, $P < 0.01$) and mortality ($r^2 = 0.42$, $F = 8.20$, $P < 0.01$; Table 2). Seed infection level was significantly and strongly related to phylogenetic distance. Species more closely related to *B. tectorum*, the host species of origin, showed lower resistance and higher infection levels. Conversely, seed mortality level (tolerance to infection) was significantly and strongly related to mean germination time. As predicted, species with longer mean germination times exhibited lower tolerance and higher mortality than species with seeds that germinated quickly. Seed hardness was significantly related to both infection and mortality, with softer seeds exhibiting higher levels. Seed size had no effect.

Pathogen density effects to predict realized host range

The proportion of seeds infected and killed in the pathogen inoculum density experiment varied by species and decreased significantly with decreasing inoculum concentration (see Table 6 in Appendix for ANCOVA). Species also varied significantly in their response to decreasing inoculum load (Fig. 2). The proportions of infected and killed *B. tectorum* seeds remained high over several inoculum concentrations and dropped below 0.50 only at levels $<10^{-3}$. Seeds of native species, in contrast, usually exhibited sharp drops, especially in mortality, at concentrations $<10^{0}$. *H. comata* seeds exhibited some pathogen-caused infection and mortality at the highest inoculum concentration but near-zero infection and mortality at all lower concentrations. *A. hymenoides* seeds displayed consistently low infection and mortality as the inoculum concentration decreased from 10^{0} to 10^{-2}, while infection and mortality in *E. elymoides* decreased with inoculum concentration over the range 10^{0}–10^{-3} (Fig. 2). *P. spicata* showed the greatest difference between infection and mortality. It had a pattern somewhat similar to *B. tectorum* for infection, while mortality decreased linearly with inoculum concentration over the range 10^{0}–10^{-3}. Reducing pathogen density reduced the realized host range relative to the potential host range by decreasing overall levels of infection and mortality. More importantly, it also narrowed the realized host range through its differential effect on species with differing levels of susceptibility and tolerance. Highly resistant species (*H. comata* and *A. hymenoides*) suffered little or no infection or mortality even at moderate inoculum loads. All four native species showed much reduced impacts, particularly mortality, at mid-level inoculum loads that caused relatively high mortality on dormant *B. tectorum* seeds.

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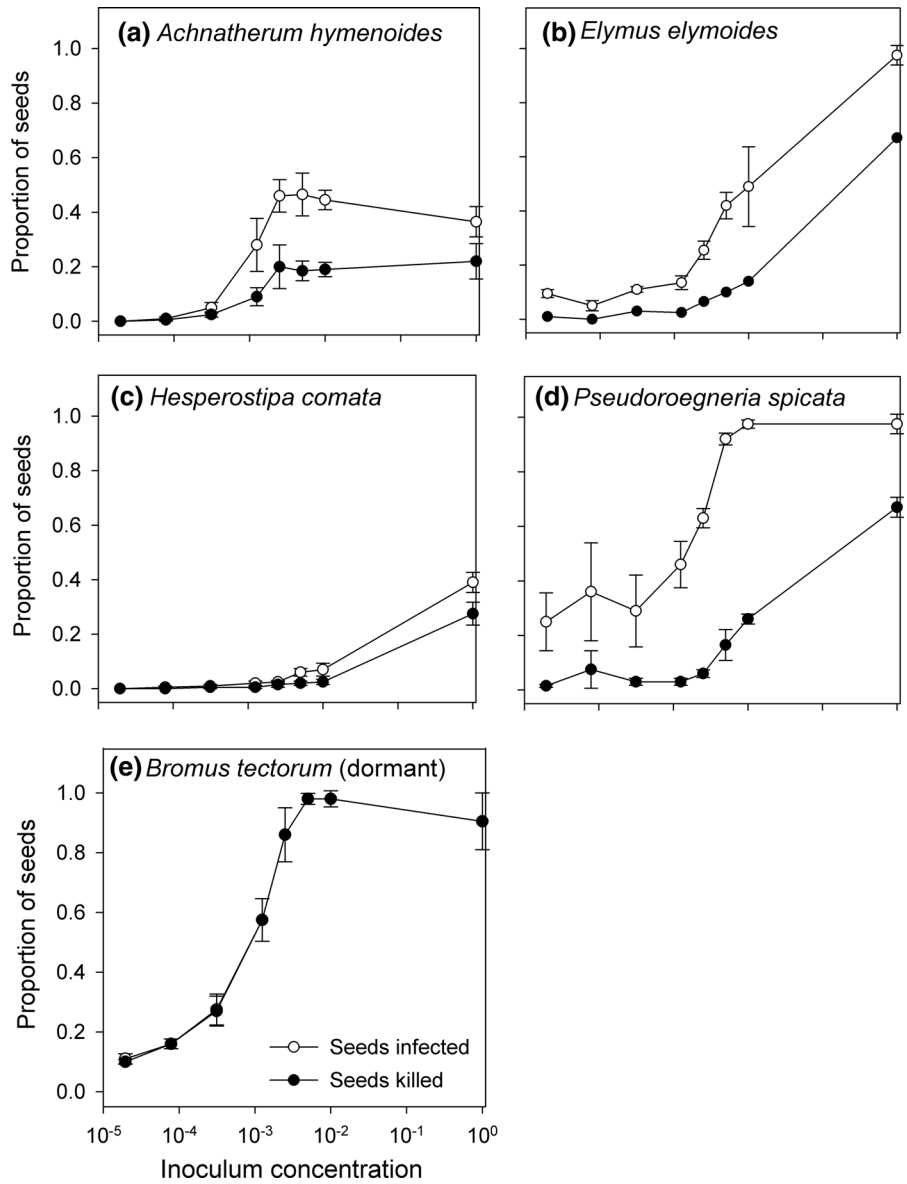

Fig. 2 Proportion of seeds infected (*white circles*) and killed (*black circles*) by *P. semeniperda* across a range of inoculum loads (note exponential scale) for five species: **a** *A. hymenoides*, **b** *E. elymoides*, **c** *H. comata*, **d** *P. spicata*, and **e** dormant *B. tectorum* (means + 1SE)

Host and pathogen density in field seed banks

Viable and pathogen-killed seed density in field in situ seed banks varied dramatically between *B. tectorum* and three native grass species across all years and all types of sites (Table 3). *B. tectorum* viable seed densities ranged from 3,040 to 10,600 per m^2, while killed seed densities ranged from 220 to 18,530 per m^2. Seed banks for native species were orders of magnitude smaller (range 0–830 per m^2) and sustained little or no seed loss to *P. semeniperda.* Only one of three native species, *H. comata*, contained pathogen-killed seeds in its seed banks, and killed seed densities were very low (two of five site-year combinations; 18 and 35 seeds per m^2). These native grass species apparently fall outside the realized host range

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Table 3 Mean densities of viable and pathogen-killed seeds (seeds, m^{-2}) in field seed banks of *B. tectorum* and three native grasses at two sites in Washington in fall 2007 and fall 2008 and at one site in Utah in fall 2007 ($n = 20$)

Study site	Year	*B. tectorum*		*E. elymoides*		*H. comata*		*P. spicata*	
		Viable	Killed	Viable	Killed	Viable	Killed	Viable	Killed
Washington xeric	2007	8,496	266	53	0	18	0	0	0
Washington xeric	2008	10,567	1,044	0	0	124	35	71	0
Washington mesic	2007	3,044	177	53	0	266	18	319	0
Washington mesic	2008	8,602	920	159	0	301	0	159	0
Utah xeric	2007	7,664	18,530	71	0	18	0	832	0

See Table 4 in Appendix for site information and Table 5 in Appendix for soil data for each site

of the pathogen, at least in the areas and years of sampling; *B. tectorum* is clearly the primary host. Even *P. spicata,* a species shown to be highly susceptible in laboratory trials, experienced no detectable mortality in the field, probably because of low seed densities.

Discussion

We have demonstrated that there is a large difference between the potential host range and the realized host range of the generalist pathogen *P. semeniperda*, and have identified several factors affecting the expression of host range in this pathogen. The measured potential host range was very broad in terms of susceptibility to infection, especially if defined simply as the number of species successfully infected (Fig. 1a). It was much narrower when defined in terms of tolerance to infection (Fig. 1b). Many species with high susceptibility (low resistance) exhibited high tolerance, so that seed mortality was low even though infection levels were high. This is similar to the pattern observed with cereal crop seeds, and which led Medd et al. (2003) to conclude that *P. semeniperda* is a weak pathogen that does little or no damage to crops. This conclusion appears to be equally valid for several perennial grass species as well as some annual grass weeds in the present study. The primary mechanism conferring tolerance to infection is rapid germination (Tables 1, 2). Seeds of *B. tectorum* were highly susceptible to pathogen infection whether dormant or nondormant, but only dormant seeds suffered very high mortality, whereas rapidly germinating nondormant seeds escaped. We hypothesize that a similar effect would be seen for other annual grasses related to *B. tectorum*, which also have high primary dormancy but rapid germination when nondormant. In contrast, grasses with persistent seed dormancy but low susceptibility (high resistance) would not be expected to exhibit much change in their relatively low mortality levels as a function of dormancy status. These more resistant species had mortality levels similar to observed infection levels, indicating low tolerance to infection.

In addition, susceptible grass species that have relatively slow germination rates even when fully nondormant would also show little change in mortality level as a consequence of dormancy loss. Species most utilized in the nondormant state and thus most at risk from this pathogen would be those with intermediate to high susceptibility of infection but slow germination rates and thus high seed mortality (i.e., low tolerance) even when nondormant.

We found host plant phylogeny to be an important predictor of susceptibility to seed infection by this pathogen. Increases in phylogenetic distance between *B. tectorum* and alternate host species corresponded with lower levels of infection by *P. semeniperda* (Tables 1, 2). This finding is similar to reports by others that phylogenetic distance helps to predict susceptibility to enemies (e.g., de Vienne et al. 2009). Our result may demonstrate a case of host-specific adaptation by particular races of the pathogen from *B. tectorum*, as suggested for other exotics (Gilbert and Parker 2010; Reinhart et al. 2011). Alternatively, it may indicate that *P. semeniperda* as a species has a limited effective host range even within grasses.

Seed hardness was a significant predictor of both susceptibility to infection and tolerance to infection, with harder seeds experiencing both less infection and less mortality (Tables 1, 2). This is in contrast to the results of Pringle et al. (2007). The physical defense

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effect found in this study appears to not only limit the ability of the pathogen to enter the seed for infection but also the ability of the pathogen to kill the seed, but this could be a consequence of the fact that species with high resistance and therefore low infection levels also have low mortality levels as a result. Our findings support those of Davis et al. (2008) that physical defenses involving the seed coverings can influence fungal infections.

Clearly, the potential host range measured in our experiments is dependent on species and accessions tested, as we were only able to test a subset of all possible combinations. However, our sample size of 26 species does include most of the dominant and ecologically important grass species in the Intermountain West. The inclusion of more species, particularly dicot species, could change our concept of potential host range in this pathogen. There may also be within-species variation in host susceptibility. Similarly, our ability to generalize may be limited by our strain choices, as strains of *P. semeniperda* show considerable variation in virulence (Meyer et al. 2010), indicating additional factors that add to the complexity of describing pathogen host range.

Pathogen inoculum density was a main factor affecting realized host range in our study. Exponential reductions in pathogen density (inoculum load) resulted in much decreased levels of utilization, whether expressed as infection/sporulation or mortality, and the shape of the relationship was host species-specific (Fig. 2). Native species tended to escape mortality even at intermediate to high inoculum concentrations through resistance or tolerance, whereas dormant *B. tectorum* seeds experienced near-complete mortality at these levels. Natural inoculum loads detected using *B. tectorum* seed-zone ring studies have resulted in mortality of dormant *B. tectorum* seeds from 5 to 40 %, which would correspond to a dilution level of 10^{-3} or lower in the present experiment (S. Meyer, unpublished data). None of the native species tested were strongly negatively impacted at comparable dilution levels, suggesting that these species would be largely unaffected by *P. semeniperda* even if planted directly into a *B. tectorum* monoculture. Similarly, in a related seed-zone ring study with five important native grasses (Beckstead et al. 2010), only *P. spicata* exhibited >10 % mortality when planted into seed-zone samples from *B. tectorum* stands.

We can compare the results of this study with Meyer et al. (2014), a study that involved field inoculum augmentation with the same pathosystem. This provides us an opportunity to examine the degree to which realized host range is reduced relative to potential host range under a more realistic field scenario. Meyer and colleagues planted two dominant native grasses into *B. tectorum* monocultures with and without augmented *P. semeniperda* inoculum at two field sites with contrasting environmental conditions. At the mesic site, seed mortality increased significantly with inoculum augmentation for *P. spicata* (37 % in augmented vs. 5 % in unaugmented) but not *E. elymoides*, while emergence was not significantly affected in either species. At the xeric site, *Pyrenophora*-caused seed mortality increased with inoculum augmentation for both species (47 % in augmented vs. 22 % in unaugmented, overall), but emergence was negatively impacted only for *P. spicata* (20 % in augmented vs. 34 % in unaugmented), a species that does not naturally occur at this xeric site. These results indicate that *Pyrenophora* attacks the same slow-germinating fraction that is subject to pre-emergence mortality from other causes, including other seed pathogens. Thus in addition to pathogen inoculum loads, environmental conditions and competing pathogens act to reduce the realized host range relative to the potential host range in this pathosystem.

Overall, we found remarkably few seeds in native grass seed banks and detected very low levels of *P. semeniperda* (Table 3). These findings indicate that the pathogen is not utilizing native grasses as hosts, likely due to their low seed density. The fact that we were often unable to detect a seed bank, even soon after dispersal, suggests that seed removal and predation may be much more important for these species, whose seeds are often preferred over *B. tectorum* by granivores (e.g., harvester ants; Crist and MacMahon 1992). Competition with granivores appears to limit the realized host range of the pathogen through density-dependent effects in these native stands. In contrast, *B. tectorum* seed banks had high seed densities and high levels of *P. semeniperda*-caused disease. The interactions between granivores and *P. semeniperda* in *B. tectorum* monocultures have not been investigated, but these interactions, and their effect on added native seeds, could be quite different from those observed in native stands. Additional factors that could impact disease levels in the field

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include relations with other seed bank microbes (Schafer and Kotanen 2004), which could be competitors or hyperparasites that reduce *P. semeniperda*-caused disease (T. Davis, J. Beckstead and S. Meyer, unpublished data).

Soils data from the in situ seed bank study sites (Appendix in Table 5) showed that native sites and *B. tectorum*-infested sites from the same locality generally had similar soil physical characteristics (except for the two Whiterocks sites which were several kilometers apart). The soils of *B. tectorum*-infested sites usually had elevated organic matter content and fertility (available N, P, K) relative to soils at native-dominated sites from the same locality. This is probably related to the high litter load and rapid turnover in the soils of *B. tectorum*-dominated sites and the consequent enrichment of the surface horizon. We have found a positive association between litter load and *P. semeniperda*-killed seed density within *B. tectorum*-dominated sites (Beckstead et al. 2012). Both of these factors are related to seed production. It is therefore likely that the primary underlying causal variable that controls disease levels within *B. tectorum* stands is seed density. This is in accord with our proposed explanation above for the very low levels of *P. semeniperda*-caused disease in native seed banks in this study.

This study reveals that the potential host range of *P. semeniperda* as measured at high inoculum loads in the laboratory is potentially a poor predictor of either the current or possible future realized host range under field conditions. Similarly, most co-occurring native grass species fell within the potential host range of *P. semeniperda* in our laboratory experiment, but associated field experiments by Meyer et al. (2014) showed that even species that experienced high seed mortality with high inoculum loads in the laboratory were largely unaffected by the pathogen under field conditions for emergence. A complex combination of intrinsic and extrinsic factors led to differential levels of host tolerance and resistance that collectively underlie the expression of host specificity in the seed pathogen *P. semeniperda*.

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Appendix

See Tables 4, 5, and 6.

Table 4 Location information for study sites included in field seed bank studies (native grass species and weedy *Bromus tectorum*), including annual mean temperature and precipitation

Study site	Land ownership	State	Latitude/ longitude	Elev. (m)	Temp. (°C)	Precip. (cm)
Pinecroft (mesic)	Washington Dept. Natural Resources	WA	47°41′N, 117°14′W	614	8.0	54.9
Brown Mountain (mesic)	Private	WA	47°61′N, 117°31′W	873	7.7	54.8
Saddle Mountain (xeric)	Hanford Reach National Monument	WA	46°47′N, 119°27′W	582	10.5	19.0
Whiterocks 1 native (xeric)	Bureau of Land Management	UT	40°20′N, 112°47′W	1,438	9.9	30.4
Whiterocks 2 *B. tectorum* (xeric)	Utah Bureau of Land Management	UT	40°19′N, 112°51′W	1,600	9.2	20.3

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Table 5 Soils information (0–15 cm) for study sites in the field seed bank studies [native grass species and *Bromus tectorum* (*BRTE*)]

Study site	Soil texture			$CaCO_3$ equiv. %	pH (sat. paste)	Salinity EC (dS cm^{-1})	Organic matter %	Nitrate N (ppm)	Olsen P (ppm)	Soluble K (ppm)
	% sand	% silt	% clay							
Pinecroft native	36.7	44.2	19.1	1.21	5.78	0.5	1.2	5.4	5.1	212
Pinecroft BRTE	32.7	50.2	17.1	1.33	5.88	0.6	5.7	10.6	21.0	403
Brown Mtn. Native	44.1	42.8	13.1	2.06	6.84	0.5	2.7	9.2	12.2	64
Brown Mtn. BRTE	34.7	49.2	16.1	1.37	6.30	0.4	3.0	7.3	24.7	62
Saddle Mtn. Native	44.7	46.2	9.1	1.91	6.17	0.8	1.3	6.2	10.7	141
Saddle Mtn. BRTE	46.7	43.2	10.1	2.04	6.86	0.4	2.1	6.9	21.5	705
Whiterocks Native	64.7	23.2	12.1	12.29	7.26	0.9	1.6	6.0	15.5	196
Whiterocks BRTE	36.7	36.5	26.8	22.54	7.30	1.1	1.3	22.2	12.4	640

Table 6 Results of statistical analyses of experiments on potential host range and effects of pathogen density

Experiment/effect	df	*F* value	*P* value
ANOVA potential host range			
Inoculation treatment main effect—Infection	1	3,109.33	<0.0001
Species main effect—Infection	26	17.08	<0.0001
Species × inoculation treatment—Infection	26	5.30	<0.0001
Inoculation treatment main effect—Mortality	1	832.85	<0.0001
Species main effect—Mortality	26	29.23	<0.0001
Species × inoculation treatment—Mortality	26	16.30	<0.0001
ANCOVA pathogen density effects			
Inoculum dilution main effect—Infection	1	209.39	<0.0001
Species main effect—Infection	4	25.09	<0.0001
Species × inoculum dilution—Infection	4	3.33	0.01
Inoculum dilution main effect—Mortality	1	269.65	<0.0001
Species main effect—Mortality	4	52.97	<0.0001
Species × inoculum dilution—Mortality	4	9.76	<0.0001

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